Hygienic quality of Mbala mpinda, a fermented food formulated with local products from Congo

Abstract

This study is part of the health safety of local foods consumed in Congo. The objective was to study the hygienic quality of Mbala mpinda, a food made from cassava retted paste and peanut paste, which was formulated with maize, taro and plantain. To do this, five (5) samples of this food were prepared in the laboratory and analyzed by counting the total aerobic mesophilic flora, coliforms total and faecal bacteria, Staphylococci aureus, yeasts and molds using the serial dilution technique (NF.V 08-051-Feb. (1999), AFNOR V 08 013 and directive 2005/2073/EC). The presence of Salmonella and Shigella was also checked on specific SS medium after enrichment of the cultures. The results showed a microbial load in FMAT of 3.5.10-3, 3.1.10-4 and 1.5.10-3 CFU/g respectively for samples E1, E2, E5. This microbial load is below the threshold set by Directive 〖10〗^6 While samples E3 and E4 have zero FMAT load. All Mbala mpinda samples were free of bacteria from the total and faecal coliform groups, Staphylococcus aureus species, yeasts and molds, as well as Salmonella and Shigella. Thus, the formulated Mbala mpinda have a satisfactory hygienic quality. It is therefore important to promote this food by popularizing it among the populations.

Keywords: Hygienic quality, fermented food, germ, Mbala mpinda

Introduction

Fermented foods are of great importance in the diet, food security and social well-being of millions of people around the world. They contribute 20-40% of food worldwide (Campbell-Platt, 1994). Fermentation also contributes to the improvement of nutritional value, the development of compounds aromatics and the digestibility of the final product (Idriss et al., 2019). This method of food preservation has been used for centuries and many fermented foods are consumed around the world and each nation has its own types and/or forms of fermented foods, representing the basic diet from the raw materials available (Louembe et al., 2003). Cassava and cereals such as maize, sorghum and millet are ground and fermented to obtain non-alcoholic products (pasta and drinks) and alcoholic beverages which are known by different names in the countries of East Africa. west (Yao et *al*., 2019).

In Congo, we see the consumption of these cooked fermented products in several forms such as chikwangue (fermented cassava tuber), poto-poto (fermented corn), ntoba mbodi (fermented cassava leaves), mbala mpinda (cassava rouie paste more peanut paste) (Kobawila et al, 2003; Louembé et al., 2003). Some of these products are eaten as main courses, others as appetizers. The consumption of a foodstuff intended for human consumption cannot be done without evaluating its quality. These are nutritional, organoleptic and hygienic aspects. The latter is the subject of the study of germs of hygienic interest which are, among others: total aerobic mesophilic flora, faecal and total coliforms, Staphylococcus aureus, salmonella and shighella (Ennadir et al., 2012). It is in this context that we thought of checking the hygienic quality of these mbala mpinda. Indeed, pathogenic microorganisms (the most frequently encountered in food) belong to the group of faecal coliforms, total coliforms, Staphylococcus aureus, Shigella and Samnolla and are sometimes responsible for health problems (FAO, 1994; Capozzi et al., 2017). The mbala mpinda which is a Congolese food must respect the rules of hygiene required to preserve the health of consumers. This gap that the present study seeks to fill aims to assess the hygienic quality of formulated mbala mpinda.

Material and methods

Nature of samples and sample

Five (5) samples of Mbala Mpinda were analyzed in this study (Figure 1). The latter were prepared for the Inrsiit agro-food technology laboratory. In these preparations, in addition to the classic formulation of Mbala Mpinda composed of roui cassava paste and peanut paste, it was added separately, in the other four formulations, corn, bananas, taro and the Mixture of these three ingredients (cassava, corn and taro). These samples were packed in plastic bags then placed in a cooler and brought back to the Applied Microbiology laboratory of the National Institute for Research in Exact and Natural Sciences (IRSEN) for microbiological analyses.

(E1) **(E2)** **(E3)** **(E4)** **(E5)**

Figure 1: Different samples of Mbala mpinda analyzed in this study: (E1) mbala mpinda made from maize; (E2) cassava-based mpinda*;* (E3) mbala mpinda made from the mixture*;* (E4) mbala mpinda made from banana; (E5) mbala mpinda made from taro.

Microbiological analyzes

Preparation of inocula

The superficial and deep parts of a sample of Mbala mpinda were mixed under aseptic conditions using a sterile spatula in the sterility zone of the bunsen burner. Then, 10 g of Mbala mpinda were taken and weighed using a precision balance (GαG Electronic Scale T500, China) around the flame of the bunsen burner. Then, this mass was introduced aseptically into a 250 mL bottle containing 90 mL of physiological water sterilized by autoclaving. The mixture was homogenized for about two minutes by manual stirring. This solution constitutes the mother solution diluted to 10-1. Then, a volume of 1 mL of the stock solution was withdrawn using a micropipette and inoculated into a test tube containing 9 mL of sterile physiological water. This mixture was homogenized as before and constitutes the 10-2 dilution. From this dilution, the operation was repeated until dilution 10-5. The different dilutions obtained will be used as inocula.

Seeding and incubation

Flore mésophile aérobie totale

A volume of 100 µL of each dilution was removed using a micropipette and then deposited on the surface of the Plate Count Agar (PCA) medium (Oxoid; Basingstoke, UK). The culture dishes were incubated at 37°C for 48 h in the oven (Memmert IN 110, Germany). The inoculations were carried out in duplicate. After incubation all colonies were counted manually.

Fecal and total coliforms

For coliforms, 100 µL of each dilution was taken using a micropipette and then deposited on the surface of Violet Red Bile Agar medium (VRBA; Oxoid, Scharleau, Spain). Then, the spreading was carried out by tight streaks over the entire surface of the agar medium. The dishes thus inoculated were incubated at 44°C for faecal coliforms and 37°C for total coliforms. At the end of the incubation, only pink, red and purplish colonies were counted manually. Two boxes were inoculated by dilution.

Staphylococcus aureus

A volume of 100 µL, dilutions 10-1, 10-3 and 10-5, was spread separately in tight streaks over the entire surface of the Chapman agar, using a pipette transformed into a spreader by flambéing with a Bunsen burner. The culture dishes were incubated at 37°C for 48 hours and only the yellow colonies are considered for counting. Two boxes were inoculated by dilution.

Yeasts and molds

One hundred (100) µL of each dilution were taken using a micropipette and then deposited on the surface of the Chloramphenicol yeast glucose agar medium (NF.V 08-051-Feb.1999). Then, the inoculum was spread using a sterile Pasteur pipette transformed into a spreader. After plating, the inoculated culture dishes were incubated at 37° C. for 72 h in a Mermmert oven (IN 110, Germany). Two petri dishes were inoculated by dilution.

Salmonella and Shigella

Salmonella and Shigella were counted in terms of presence or absence (AFNOR V 08 013 standard). For this, the inoculations were carried out as follows: A mass of 25 g of each sample of Mbala mpinda was weighed on a precision balance as before and inoculated into 100 mL of the pre-enrichment broth. After incubation of this first culture, 1 mL is introduced into 10 ml of Rappoport broth in a tube. This second culture is incubated for 24 h. At the end of this second incubation, 100 µL are taken using a micropipette and then spread on the surface of the Salmonella and Shigella (SS) agar medium using the dial spreading method. The plates thus inoculated were incubated for 24 hours at 37°C.

Bacteria Reading

At the end of the incubation, the culture dishes containing a number of colonies less than or equal to 300 were selected for the manual counting of the colonies. After counting, the number of bacteria or germs (N) in colony forming units (CFU) per gram of sample was calculated according to the following formula:

$$\mathrm{N}\ (\text{CFU/g of sample}) = \frac{n}{V_{I.D}}\ X\ \frac{V_{SM}}{V_M}$$

N= Number of bacteria CFU/g
n= Colony average of the considered dilution
V= inoculum volume
D= dilution factor
V_{SM}= stock solution volume
V_M= Sample mass

Data processing

The data was processed using GraphPad Prism 7.00 for graphical representation and the between-sample similarity dendrogram was performed on Past 3.26 using UPGMA matching algorithm and Bray-Curtis similarity index with boot N from 1000.

Results

Total aerobic mesophilic flora (FMAT)

Figure 1 shows the number of microorganisms of the total aerobic mesophilic flora in the 5 samples of Mbala mpinda. Samples E1, E2, E5 have a microbial load of 3.5.10-3, 3.1.10-4 and 1.5.10-3 CFU/g of sample respectively, while samples E3 and E4 have a zero FMAT load. i.e. does not contain microorganisms of this group. All these samples have a FMAT load below the threshold value set by Directive 2005/2073/EC.

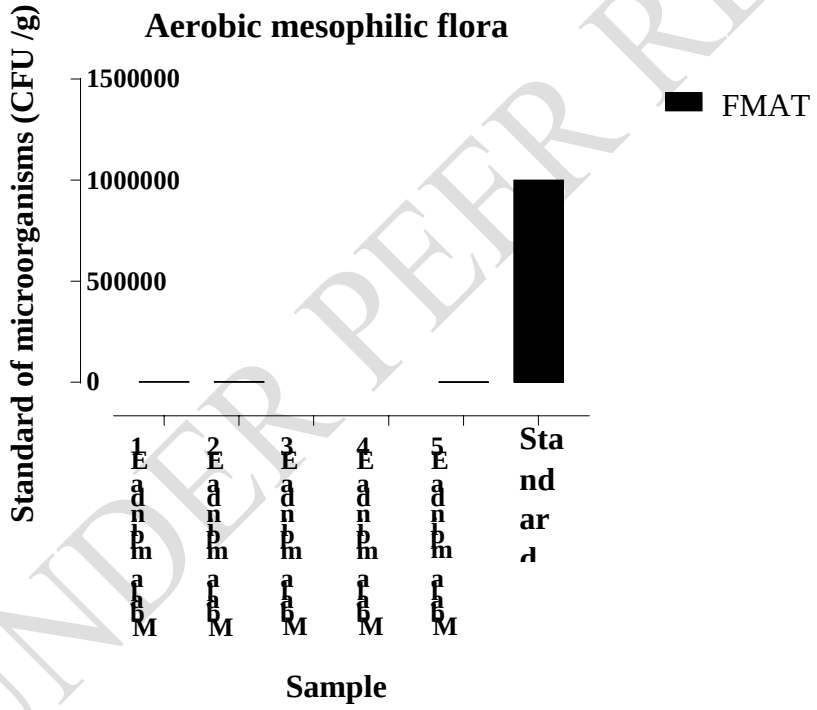

Figure 2: Enumeration of total aerobic mesophilic flora

III.2. Faecal and total coliform load

None of the samples analyzed contains bacteria belonging to the group of total coliforms or faecal coliforms (Table I). Thus, the microbial density complies with the threshold values of directive 2005/2073/EC which are 10 and 10+3 CFU/g respectively for faecal coliforms and total coliforms.

Table I**:** Enumeration of faecal and total coliforms

Sample	Faecal coliforms (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)	Total coliforms (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)
Mbala mpinda E1	0	10^1	0	10^{+3}
Mbala mpinda E2	0		0	
Mbala mpinda E3	0		0	
Mbala mpinda E4	0		0	
Mbala mpinda E5	0		0	

Staphylococcus aureus load

Table II presents the results of the enumeration of bacteria belonging to the species Staphylococcus aureus. All samples analyzed are free of Staphylococcus aureus. And these samples comply with directive 2005/2073/EC. This shows their good quality in relation to this microbiological parameter.

Table **II**: Enumeration of Staphylococcus aureus in the 5 samples of Mbala mpinda

Sample	Staphylococcus aureus (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)
Mbala mpinda E1	0	10^{+2}
Mbala mpinda E2	0	
Mbala mpinda E3	0	
Mbala mpinda E4	0	
Mbala mpinda E5	0	

Fungal load

Table III shows the load of fungi (yeasts and moulds) in the 5 samples of Mbala mpinda. All samples tested are yeast and mold free.

Table **III**: Enumeration of yeasts and molds in the 5 samples of Mbala mpinda

Sample	yeasts and molds (CFU/g of sample)
Mbala mpinda E1	0
Mbala mpinda E2	0
Mbala mpinda E3	0
Mbala mpinda E4	0
Mbala mpinda E5	0

Salmonella and Shigella

None of the five samples of Mbala mpinda analyzed showed colonies of Salmonella or Shigella after inoculation of the enrichment cultures on specific SS medium (Table IV).

Table IV: Results of analyzes of Mbala mpinda samples in terms of absence or presence of Salmonella or Shigella

Sample	***Salmonella***	***Shigella***	Standard
Mbala mpinda E1	Absence	Absence	Absence / 25 g
Mbala mpinda E2	Absence	Absence	
Mbala mpinda E3	Absence	Absence	
Mbala mpinda E4	Absence	Absence	
Mbala mpinda E5	Absence	Absence	

Hierarchical classification

Figure 3 shows the similarity dendrogram between samples. The latter was carried out from the results of the count. The samples were thus grouped into two classes based on their microbial load. A class formed by the couple Mbala mpinda E3 and Mbala mpinda E4 which are free from all microbial contamination with a similarity index of more than 0.9. And a second class formed by the rest of the samples. In this second class, samples E5 and E1, with a load of about 10+3 CFU/g, form a cluster (similarity index of about 0.6) associated with sample E2 whose microbial density is of the order of 10+4 CFU/g.

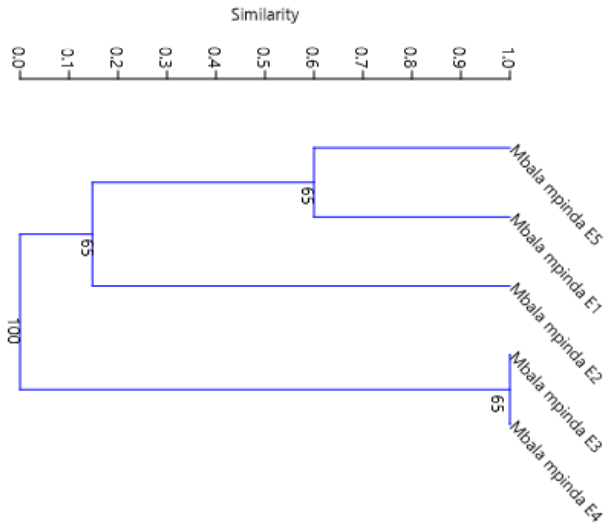

Figure 3: Dendrogram of similarity between samples

Discussion

Fermented foods, whether consumed after cooking or not, are the basis of the diets of populations in developing countries (Lawane et *al*., 2019). This is because of the selling price of these foodstuffs within the reach of low-income populations. However, the lack of control of the fermentation process means that the microbiological and nutritional quality of the final product is fluctuating and is not always satisfactory (Maïwore et *al*., 2018; Katinan et *al*., 2012; Louémbé et *al*., 2003). However, the consumption of a foodstuff intended for human consumption cannot take place without assessing its quality. These are nutritional, organoleptic and hygienic aspects. The objective of this work was therefore to assess the hygienic quality of Mbala mpinda, a fermented food consumed in the Republic of Congo. The results of the microbiological analyzes showed that all the samples had an FMAT load below the threshold value set by Directive 2005/2073/EC. Indeed, samples E1, E2, E3, E4 and E5 have an FMAT load of between 0 and 104 CFU/g of sample. Ennadir et *al* (2012) found an average load of 4 × 104 CFU/g and 2.5 × 104 CFU/g respectively in traditional and industrial wheat flours. Nevertheless, the values in the study by these authors are also below the

threshold value set by the Directive. This low density microbial could be explained by the compliance with good preparation or manufacturing practice. When these rules are not followed, it could cause an abundance of total mesophilic aerobic flora. According to Maïwore et *al* (2018), the reasons for this abundance of FMAT are: the unhealthiness of the product manufacturing environment and the utensils used; non-compliance with hygiene rules during the transformation of the raw material into a product; gales, dust and flies that settle on the ladle used as a measure during the sale, in the case of liquid products sold without air conditioning.

The count of bacteria belonging to the group of total and faecal coliforms did not reveal the presence of any colony of these bacteria in all the samples of Mbala mpinda analyzed. Thus, the microbial density complies with the threshold values of Directive 2005/2073/EC which are 10 and 10+3 CFU/g respectively for faecal coliforms and total coliforms. Our results are similar to those of Yeo et *al* (2021) who did not find total or faecal coliforms in tomato purees. The absence of bacteria from the coliform group could be explained by the fact that cooking Mbala mpinda already packaged would destroy these microorganisms. The same is true for bacteria belonging to the species Staphylococcus aureus. Indeed, these samples are also free of these germs and therefore comply with Directive 2005/2073/EC. It is clear, as pointed out by Gagara et *al* (2022) and Dieng (2001), that poor hand hygiene, the manufacturing environment, defective or contaminated equipment are likely sources of contamination. When these sources of contamination are controlled and mastered, the final product will be of very good hygienic quality as in the case of this study.

With regard to yeasts and moulds, the 5 samples of Mbala mpinda tested are free of these fungi. Our results differ from those of Anoman et *al* (2018) and Achouke et *al* (2018) who worked respectively on a food called garba and corn bread or kandji. This difference could be explained by the fact that Mbala mpinda is a packaged food and therefore protected from any external contamination. Par contre, les deux aliments analysés dans les études d'Anoman et *al* (2018) et Achouke et *al* (2018), bien que emballés, pendant la préparation, ils sont exposés et vendus dans les rues après avoir été déballés. Ainsi, ces aliments sont à la portée de tous les aérosols présents dans l'air ambiant. On the other hand, the two foods analyzed in the studies of Anoman et *al* (2018) and Achouke et *al* (2018), although packaged, during preparation they are displayed and sold in the streets after being unpacked. Thus, these foods are within reach of all the aerosols present in the ambient air.

Finally, the search for Salmonella sp. and Shigella sp. in enrichment cultures by inoculation on specific SS medium showed a total absence of these germs in all samples of Mbala mpinda analyzes. Our results corroborate those of Baba-Moussa et *al* (2006). These authors had analyzed street foods in Cotonou. This absence of these pathogenic bacteria could be explained by good manufacturing practices such as sanitation of the food preparation environment and compliance with aseptic rules.

Conclusion

This work aimed to assess the hygienic quality of Mbala mpinda and these formulations by incorporating plantain, taro and corn. The results of the microbiological analyzes show that these samples comply with Directive 2005/2073/EC. They are therefore of very satisfactory microbiological quality and likely to be subjected to sensory analysis. Salmonella sp. being a very dangerous germ responsible for typhoid fever, its presence in the samples would have raised serious questions. The absence of these germs in these samples of Mbala mpinda validates the manufacturing process and the formulations obtained.

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